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MAPPING AND CLONING OF BLACKLEG RESISTANCE GENES IN BRASSICA SPP

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Project: Mapping and cloning of blackleg resistance genes in *Brassica* spp

Abstract

Two genes for resistance to blackleg caused by *Leptosphaeria maculans* were identified in two *Brassica napus* doubled haploid (DH) populations screened for resistance in Australian disease nurseries. The genes were derived from *B. sylvestris* (a wild relative of *B. rapa*) and designated as *LepR1* and *LepR2* which mapped to linkage group N2 and N10, respectively. *LepR1* and *LepR2* control both cotyledon and adult resistance. Populations for high resolution mapping of the genes were developed and evaluated for cotyledon resistance using specific isolates recognized by the genes. Informative recombinants were identified by examination of BC1 individuals using flanking microsatellite markers for *LepR1* or *LepR2*. The region closely flanking *LepR1* located on linkage group N2 is associated with a collinear region on Chromosome 1 of Arabidopsis. Physical mapping of *LepR1* has commenced by screening a *Brassica napus* BAC library. However, *LepR2* confers partial resistance and is incompletely dominant to isolates 86-12, 89-21 and WA51 that were used for cotyledon resistance screening in the BC1 population for *LepR1*. This made it impossible to score the phenotype of *LepR2* in a BC1 population. The original populations were then screened with a number of pathogen isolates to identify a pathotype (isolate 87-41) which was avirulent on N10 resistant lines. *LepR2* confers a dominant resistance to 87-41. This has allowed high resolution mapping of *LepR2* in the BC1 population to proceed also.

Both *LepR1* and *LepR2* resistance was associated with callose deposition. *LepR1* could prevent hyphal penetration, while *LepR2* reduced hyphal growth and inhibited sporulation in cotyledons. DH lines that carry both R loci, *LepR1LepR2*, were developed to investigate epistatic gene interactions between *LepR2* and *LepR2*. Based on cotyledon disease rating the two genes interact synergistically to elicit increased resistance against *L. maculans* with additive effects.

Executive Summary

Two new genes, which confer a high degree of resistance to the stem canker phase of blackleg and are effective against all isolates of the pathogen, have been identified in materials obtained from Advanta Seeds Canada. These materials consisted of two DH populations of *B. napus* with blackleg resistance introgressed from *B. rapa* var. *sylvestris*. Lines were evaluated for resistance in field blackleg nurseries in Australia where blackleg disease severities are high and a broad range of pathotypes of *L. maculans* occur. Using RFLP and microsatellite markers, we have mapped two previously unknown genes. One on linkage group N2 (*LepR1*) was identified from the first population, and the second on linkage group N10 (*LepR2*) was identified from the second population. Lines with one or other of the two genes have been characterized against a range of isolates of *L. maculans*. Although, both resistance genes confer complete resistance at the adult plant stage, seedling resistance, conditioned by the same loci, appears to be race specific. At the cotyledon stage *LepR1* (the gene on N2) seems to confer stronger resistance than *LepR2* on N10 to most isolates tested. Crosses to Westar with lines with *LepR1* or *LepR2* and also between lines with *LepR1* and *LepR2* were made to analyze the inheritance of the resistance at the cotyledon stage. High resolution mapping of *LepR1* for the purpose of map based cloning of the genes is in progress for both *LepR1* and *LepR2*, using collinearity with the Arabidopsis sequence, RFLP

markers, and microsatellite markers. The region close to the N2 gene has been shown to be collinear with a region of 500 kb on chromosome 1 of Arabidopsis. Further informative recombinant lines in the BC1 populations have been identified to shorten the interval containing *LepR1*. Screening the *B. napus* BAC library has commenced, using flanking *LepR1* RFLP markers as probes.

Technical Report

Objectives

The objectives of the project were:

1. to obtain markers tightly linked to resistance genes for marker assisted breeding program.
2. to determine the genetic basis for the resistant reaction of lines with the *LepR* gene(s).
3. to develop high resolution mapping of the *B. sylvestris* R gene(s) and to clone the genes by map based cloning methods.

Materials

Two DH populations, 95-0165 and 96-0209, were provided by Advanta Canada Inc. The DH populations consisted of 16 resistant (R) and 15 susceptible (S) lines for 95-0165 and 15 R and 15 S lines for 96-0209. These populations were used for preliminary mapping. Further materials for further genetic analysis and for high resolution mapping were derived from these starting materials by crossing breeding. For genetic analysis of *LepR1* and *LepR2*, F1, F2, BC1 and a test cross between Westar × F1 were developed; for high resolution mapping, Westar × *LepR1* line and Westar × *LepR2* line crosses were made. F1, F2 and BC1 were developed for each cross. In addition, populations of Westar × (DH12075 × *B. sylvestris*) for mapping, germplasm development, and to search for other R genes effective against *L. maculans* were made. Populations for mapping *B. rapa* resistance genes derived from *rapa* × *sylvestris* were also developed. F1, F2 and BC1 were developed. A DH population of *LepR1* × *LepR2* for estimating the effect of pyramiding *LepR1* and *LepR2* genes on field resistance to blackleg was developed and will be tested in Australia in 2002. Populations of Westar × Surpass 400 were developed to map the resistance gene(s) in Surpass 400. F1, F2 and BC1 were made.

Pathogen isolates (*Leptosphaeria maculans*) isolates 86-12 (pathogenicity group (PG) 2); 87-41 (PG2); 89-21 (PG3), WA51 (PG4) and another 20 isolates were used for indoor screening and for evaluation of race specificity of the disease reaction phenotype of plant materials.

Experimental

Propagation of DH lines and marker analysis

Three plants of each Advanta DH line were selfed for seed production. Leaf tissue from these same plants was collected and DNA extracted from the tissue. Identification of the resistance gene(s) was initiated using RFLP markers and further confirmed using microsatellite markers. Each polymorphic restriction fragment or microsatellite band was considered as a unit character and scored as presence (+) or absence (-) of the band. The linkage of blackleg resistance in the DH populations to DNA markers was tested with Mapmaker, version 3.0 (Lander et al 1987), using Kosambi's mapping function (Kosambi, 1944) and a log-likehood threshold of 4.0. Analysis of microsatellite markers was performed in populations (Westar x *LepR1*) BC1 (46 individuals), (Westar x *LepR2*) BC1 (46), (*LepR1* x *LepR2*) DH (78), (Westar x Surpass 400) BC1 (138) and (Westar x triploid) BC1 (112).

Evaluation of disease reaction phenotypes

Seed from the increased lines was planted and seedlings evaluated for cotyledon reaction and adult plant resistance to different pathotypes of *Leptosphaeria maculans* for dissection of resistance genotypes and phenotypes. Standard inoculation and incubation procedures were used.

Plants from a line from DH population 95-0165 (*LepR1*), from 96- 0209 (*LepR2*), lines from the DH population of *LepR1* x *LepR2*, Surpass 400 (*B. napus* cultivar carrying *B. rapa, sylvestris* R gene), DH12075 (N7 R line) and W12D1 (*B. napus* line containing R gene from *B. juncea*) were evaluated for cotyledon resistance to 20 *L. maculans* isolates that originating from Australia, Europe, United States and different regions of Canada. The purpose of this was to investigate the specificity of the resistance conferred by different R genes, epistatic gene interaction between *LepR1* and *LepR2*, and the relationship between Surpass 400 R gene and *LepR1* or *LepR2*.

Plants for analysis of DNA markers above were tested for cotyledon disease phenotype (resistance or susceptibility) to isolate 86-12, or WA51, or 87-41 or both WA51 and 87-41.

Construction of backcross populations

Three resistant lines per DH population were crossed with a DH susceptible line (Westar N-0-101). Resistance of F1 and parents was evaluated with different pathotypes of *L. maculans*. Two BC populations each with more than 10,000 seeds were developed between a recurrent parent (Westar) and F1 hybrids to generate large numbers of meiotic recombinants in the region of the disease resistance loci for high resolution mapping and positional cloning of the resistance genes.

High resolution mapping

BC1 and F2 populations from Westar × *LepR1* line and Westar × *LepR2* line have been obtained for high-resolution mapping (10,000 BC1 lines). Since the resistance genes *LepR1* and *LepR2* were derived from *B. sylvestris*, a triploid population (*B. napus* × *B. sylvestris*) was made for further confirmation of the presence of resistance genes using microsatellite markers and to develop resistant lines for plant breeding purposes. High resolution mapping in order to clone the resistance genes from *B. sylvestris* was initiated in January 2001 using the Westar × *LepR1* BC1 populations. We have also identified two pathotypes of *L. maculans*, which can be used in cotyledon assays to distinguish *LepR1* and *LepR2* resistance and susceptibility phenotypes rapidly and accurately. Flanking microsatellite markers (within 10 centimorgans) have been identified for each resistance gene.

Project Developed Materials:

Populations for high resolution mapping of Westar × *LepR1* line and Westar × *LepR2* line. (F1, F2 and BC1)
Populations for genetics analysis of *LepR1* × *LepR2*. (F1, F2 and test cross between Westar × F1)

Populations of Westar × (DH12075 × *B. sylvestris*) for mapping, germplasm development, and to search for other R genes effective against *L. maculans*.
Populations for mapping *B. rapa* resistance genes derived from *rapa* × *sylvestris*. (F1, F2 and BC1)
DH population of *LepR1* × *LepR2* for pyramiding the two resistance genes.
Populations of Westar × Surpass 400 for mapping resistance gene. (F1, F2 and BC1)
Differential pathotypes of *L. maculans* for discrimination of *LepR1* and *LepR2* resistance genes.
Preliminary results of this work were presented by SR Rimmer at the Intl. Symposium on Rapeseed Science, Wuhan, PRC, April 19-23, 2001.
Yu FQ, SR Rimmer, DJ Lydiate & G. Buzza. 2002. Identification of two loci in Brassica napus for resistance to Leptosphaeria maculans PAG X, San Diego. Poster presented January 2002.
Manuscript on identification, mapping and characterization of *LepR1* and *LepR2* has been prepared
Manuscript on differentiation between *LepR1* and *LepR2* using isolates of *L. maculans* is in preparation.

Project Photos: None at this time.

Publications:

Yu, FQ, DJ Lydiate, G Buzza and SR Rimmer (2002) Identification of two loci in *Brassica napus* for resistance to *Leptosphaeria maculans* (in preparation for Plant Journal)

Yu FQ, SR Rimmer, DJ Lydiate & G Buzza. (2002). Identification of two loci in *Brassica napus* for resistance to *Leptosphaeria maculans*. Poster at Plant and Animal Genome X, San Diego CA. January.

Rimmer SR, FQ Yu, G Ardiel, and DJ Lydiate. 2002. Mapping resistance genes in *Brassica napus* to *Leptosphaeria maculans*. Canadian Phytopathological Society Meeting, Waterton, AB. June.

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